On peroxidase activity of Glutathione Peroxidase

FACULTAT DE CIÈNCIES, TECNOLOGIA I ENGINYERIES
UVIC | UVIC·UCC

Bi-squared Research Group Bioinformatics and Bioimaging UVIC·UCC

Nayanika Das [1#] , Martin Floor Pilquil[2#] , Vijay Baladhye[3] , Jordi Villà-Freixa[1*]

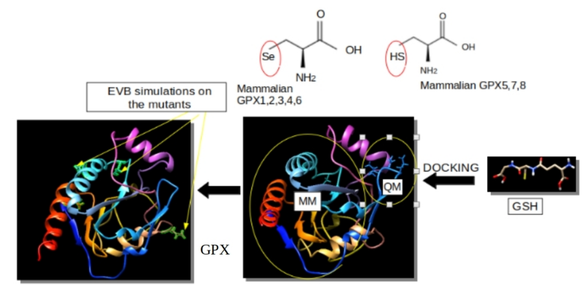
1.Research Group on Bioinformatics and Bioimaging (BI2); Universitat de Vic - Universitat Central de Catalunya/Institut de Recerca i Innovació en Ciències de la Vida i de la Salut a la Catalunya Central

2.Barcelona Supercomputing Center, Barcelona, Spain; 3.Bioinformatics Centre, Savitribai Phule Pune University, Pune, Maharashtra, India

(#) The authors contributed equally to the work.

Abstract

The biological effects of selenium are largely mediated by selenium-containing proteins (selenoproteins) [1]. Selenoproteins are broadly divided into three families such as Glutathione peroxidases (GPXs), Thioredoxin reductases (TRs) and Iodothyronine deiodinases (DIOs) [2] [3].Most of the identified mammalian GPX are selenoproteins and employ a selenocysteine (SeCys or Sec) in place of Cys at the catalytic site.[4].GPX is part of the glutathione REDOX system and converts glutathione to its oxidized form, thereby reducing H_2O_2 to water, and lipid hydroperoxides to their corresponding stable alcohols. [5]. To maintain a low level of ROS production, tissues are equipped with a robust antioxidant defense system that includes endogenous glutathione, enzymatic antioxidants (e.g., superoxide dismutase: SOD, catalase: CAT, and glutathione peroxidase: GPX) [6] .Previous studies have identified multiple GPX-like peroxidases in diverse organisms, including fungi, plant, insects, rodents,bacteria in which the GPX-like proteins exhibit significant sequence homologies. Se-supplementation has an impact on the gut microbiota, suggesting a key role between Se-microbiota and human health. [7] Our preliminary results show that catalytic activity of several reconstructed ancestral structures of GPX6 recover their peroxidase activity when the active site is mutated from Cys to Sec keeping the binding of glutathione in all cases. We will focus on understanding both phylogeny and enzymatic mechanism in GPX (Sec- or Cys-containing), and our final goal is to understand the effect of mutations in the enzymatic mechanism. To achieve this, we make use of free energy calculations in GPX and its orthologs.



QUESTION

How is the interplay of gut microbiota and oxidative stress associated with accumulation of mutations in GPX in intestinal disease.

OBJECTIVES

GPX6 is our model protein because of the existence of Cys and Sec-containing homologs

DONE: 1. To explore the mode of interaction between GPX6 and the glutathione cofactor in GPX.

IN PROGRESS:

2. corroborate the GPX6 peroxidase enzymatic mechanism (using QM/MM methods)

3. Explore the role of GPX6 interspecific variants in the peroxidase activity (using empirical valence bond -EVB- calculations)

4. Explore the molecular evolution pathways for the differentiation of Cys/Sec GPX6 homologs

HYPOTHESIS

We are interested in understanding the role of presence of selenocysteine in GPX isoforms within their metabolic context

Introduction

1

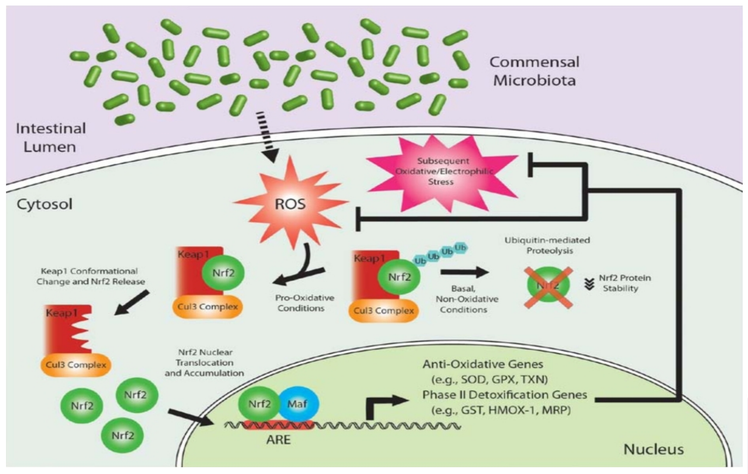
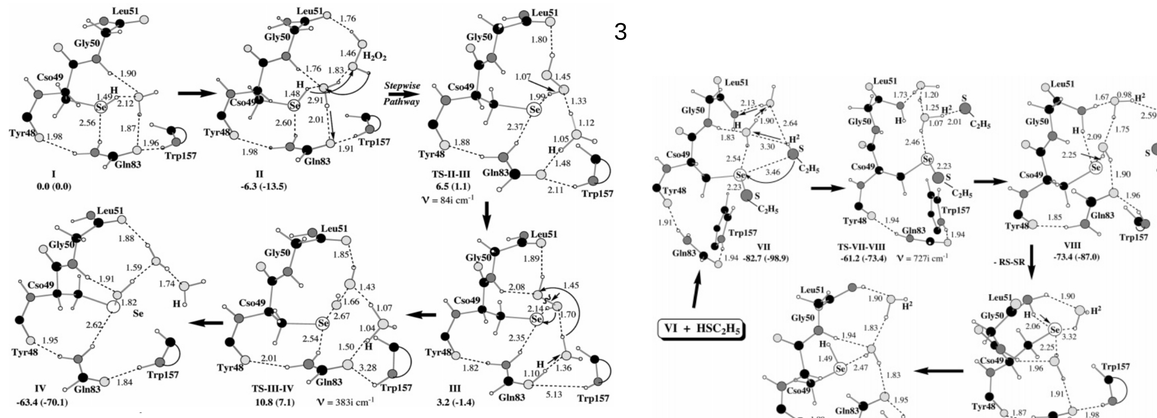


Fig 1 . Activation of the Keap1/Nrf2/ARE signaling module through microbe-induced ROS generation [9]



3

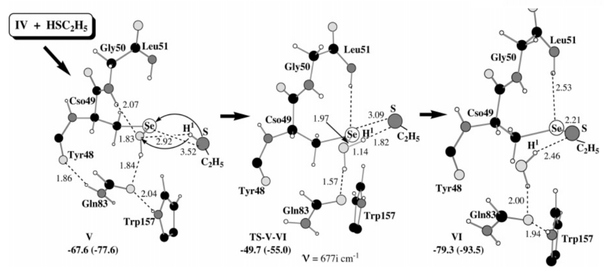


Fig 3. Optimized structures and energies in kcal/mol] of the reactant, intermediates and TS for the stepwise mechanism of Bovine GPX [2]

Fig 1. Generally accepted that Nrf2-regulated genes fall into at least 2 categories,
1. The antioxidant enzymes, which include superoxide dismutase (SOD), glutathione peroxidise (GPX) and thioredoxin (TXN)
2. Detoxification enzymes, such as glutathione S-transferase (GST), heme oxygenase-1 (HMOX1) and multidrug resistance-associated proteins (MRP's) [9]

2

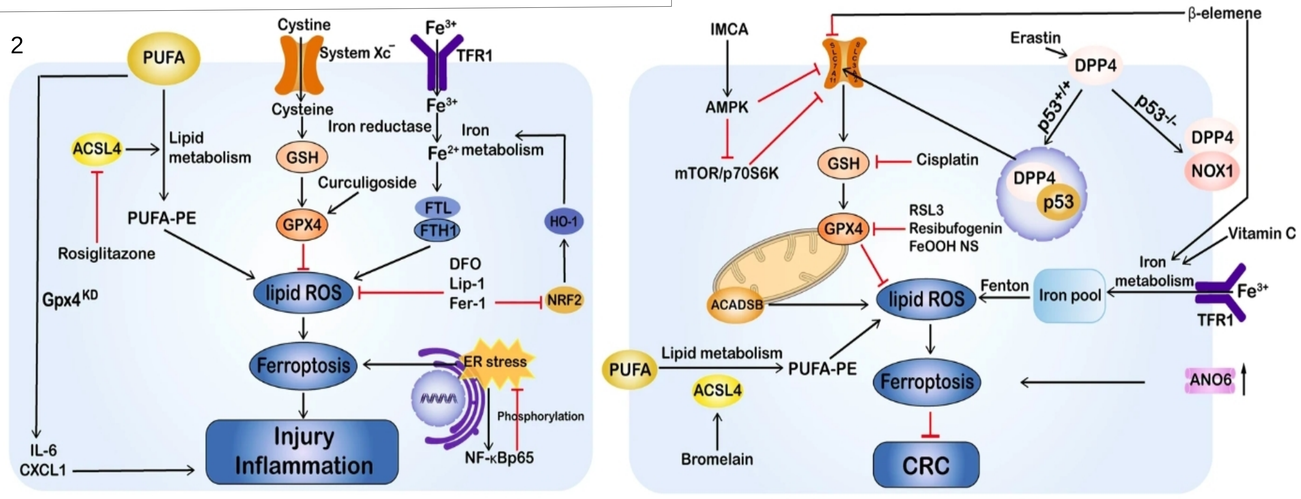


Fig 2. The common ferroptotic mechanisms in intestinal diseases include GPX4 inhibition. Key regulators such as GPX4, SLC7A11, ACSL4, and p53 are also important for mediating ferroptosis-associated intestinal diseases [8]

4

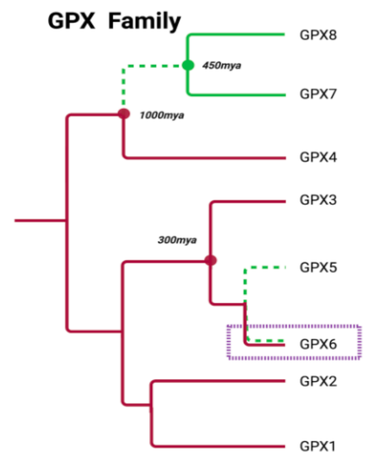
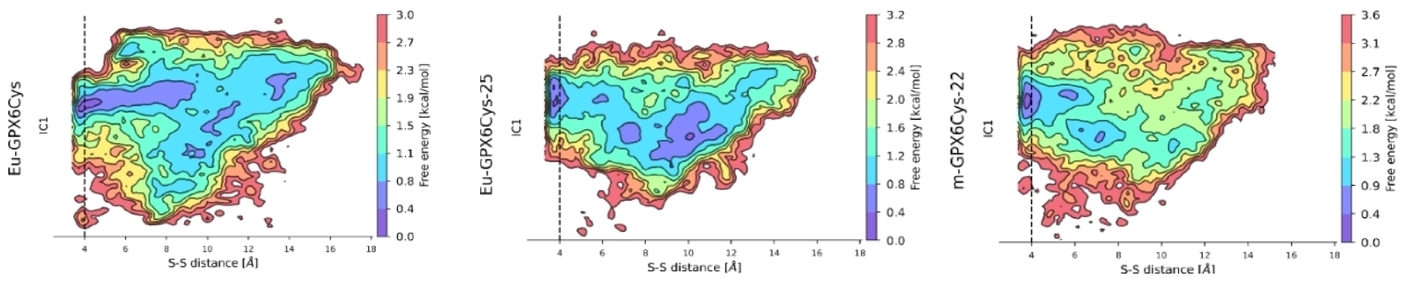


Fig 4.The phylogeny of the GPX family in Eukaryotes (based on Mariotti et al., 2012). In red, GPX6 Sec branches. In green, GPX6Cys ones. Dashed green branches represent GPX6 Cys lineages where Sec was lost. [3]

Results and Conclusion

5



6

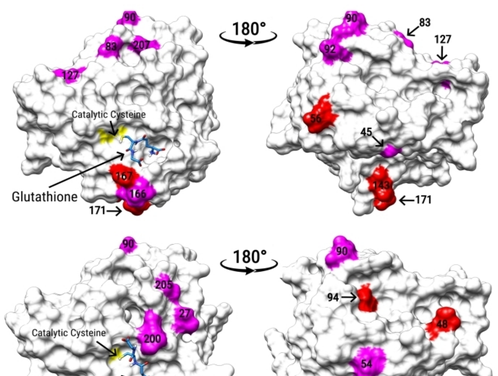
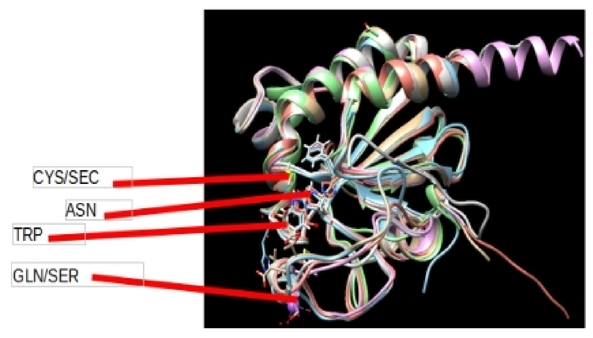


Fig 5. Computational analysis suggests that the binding of GSH and overall structures of the enzymes have not been adversely affected by the involvement of Cys.
Fig 6.The conservation of GPX6-glutathione interactions, despite the loss of peroxidase activity may suggest additional functional roles of GPX6 still to be described. [3]

7



8

Fig 5- Free energy profiles for the docking of the glutathione dimer to ancestral and modern GPX6 Cys proteins.
Fig 6- Convergence patterns from Eu-GPX6Cys to Eu-GPX6Cys+25 (top) and from Eu-GPX6Cys+25 to m-GPX6Cys+22 (Mouse-GPX6) (bottom). The catalytic cysteine (yellow) is shown with the glutathione best binding energy conformation.
Fig 7-Structural homology in Mammalian GPX showing the catalytic residues
Fig 8-RMSF plot of GPX6 human wild type

Methods

Current status

1.Alphafold2 for protein structure reconstruction from ancestral sequences.
2.Molecular dynamics simulations (OpenMM, AMBER ff) to explore the conformational landscapes of GPX6 and its complexes with glutathione disulfide
3.MDTraj to create the RMSF plots.
4.Protein-ligand binding energy landscape explorations was done using the PELE software (Protein Energy Landscape Exploration)

References

1. Sznarkowska A, Kostecka A, Meller K, Bielawski KP. Inhibition of cancer antioxidant defense by natural compounds. Oncotarget. 2017 Feb 28;8(9):15996-16016. doi: 10.18632/oncotarget.13723. PMID: 27911871; PMCID: PMC53
2. Prabhakar R, Vreven T, Morokuma K, Musaev DG. Elucidation of the mechanism of selenoprotein glutathione peroxidase (GPx)-catalyzed hydrogen peroxide reduction by two glutathione molecules: a density functional study. Biochemistry. 2005 Sep 6;44(35):11864-71. doi: 10.1021/bi050815q. PMID:16128588.
3. Ancient loss of catalytic selenocysteine spurred convergent adaptation in a mammalian oxidoreductase. Rees JSarangi GCheng QFloor MAndrés AMiguelBVillà-Freixa JSj Arnér ECastellano S. Doi: 10.1101/2023.01.03.522577
4.Bao YJ, Zhou Q, Yu X, Yu X, Castellino FJ. Analysis and Characterization of Glutathione Peroxidases in an Environmental Microbiome and Isolated Bacterial Microorganisms. J Microbiol Biotechnol. 2023 Mar 28;33(3):299-309. doi: 10.4014/jmb.2209.09006. Epub 2023 Jan 20. PMID: 36788458; PMCID: PMC10084747.
5.Shandilya S, Kumar S, Kumar Jha N, Kumar Kesari K, Ruokolainen J. Interplay of gut microbiota and oxidative stress: Perspective on neurodegeneration and neuroprotection. J Adv Res. 2021 Sep 17;38:223-244. doi: 10.1016/j.jare.2021.09.005. PMID: 35572407; PMCID: PMC9091761.
6.Ayemele AG, Tilahun M, Lingling S, Elsaadawy SA, Guo Z, Zhao G, Xu J, Bu D. Oxidative Stress in Dairy Cows: Insights into the Mechanistic Mode of Actions and Mitigating Strategies. Antioxidants (Basel). 2021 Nov 29;10(12):1918. doi: 10.3390/antiox10121918. PMID: 34943022; PMCID: PMC8750585.
7. Ramírez-Acosta S, Selma-Royo M, Collado MC, Navarro-Roldán F, Abril N, García-Barrera T. Selenium supplementation influences mice testicular selenoproteins driven by gut microbiota. Sci Rep. 2022 Mar 10;12(1):4218. doi: 10.1038/s41598-022-08121-3. PMID: 35273298; PMCID: PMC8913620.
8. Xu S, He Y, Lin L, Chen P, Chen M, Zhang S. The emerging role of ferroptosis in intestinal disease. Cell Death Dis. 2021 Mar 17;12(4):289. doi: 10.1038/s41419-021-03559-1. PMID: 33731703; PMCID: PMC7969743.
9. Jones RM, Mercante JW, Neish AS. Reactive oxygen production induced by the gut microbiota: pharmacotherapeutic implications. Curr Med Chem. 2012;19(10):1519-29. doi: 10.2174/092986712799828283. PMID: 22360484; PMCID: PMC4269156.